

Effect of amlodipine on blood glucose level in euglycemic and streptozotocin induced diabetic Albino rats and its pharmacodynamic interaction with glibenclamide

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ABSTRACT

Background: Diabetes is one of the largest global health emergencies of the 21st century and its co-existence with hypertension is frequent. These conditions often require polypharmacy with possible risk of drug interaction. This study is conducted to investigate the effect of amlodipine on blood glucose level in euglycemic and diabetic rats and its pharmacodynamic interaction with glibenclamide.

Methods: Rats were divided into six groups of 6 rats in each group. Group 1 and 3 were non-diabetic given 1% Gum acacia and amlodipine respectively. Group 2, 4, 5 and 6 were made diabetic by using nicotinamide and streptozotocin injection intra peritoneally and given 1% Gum acacia, glibenclamide, amlodipine and amlodipine + glibenclamide respectively for the period of 28 days. Fasting Blood Glucose (FBG) levels were measured before induction of diabetes, 72 hrs after the induction, on day 0, 7th, 14th, 21st and 28th day.

Results: Amlodipine produced no significant effect on FBG level in non-diabetic rats but in diabetic rats statistically significant hyperglycemia were observed on day 21st and 28th of study with the 'p' value (<0.05). Glibenclamide treated rats shows better controlled FBG level throughout study than concomitant administration of glibenclamide with amlodipine. Significant rise in blood FBG level with 'P' value (<0.05) were observed in amlodipine + glibenclamide treated group on 21st and 28th day of study.

Conclusions: This study suggest amlodipine produce no effect on the FBG level of normal rats but causes significant hyperglycemia in diabetic rats. Hypoglycemic effect of glibenclamide gets blunted when co-administered with amlodipine.

Keywords: Amlodipine, Diabetes, Drug interactions, Glibenclamide

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in Insulin secretion, Insulin action or both.¹ It is a multifactorial disorder. Among the different types the Type 2 Diabetes has most frequent occurrence and consists of about 90% of all the cases of diabetes.² According to the International Diabetes Federation (IDF) diabetes is one of the largest global health emergencies of the 21st century. The number of people living with diabetes worldwide is approximately 415 million in 2015 and is expected to rise to 642 million by 2040.³

The co-existence of hypertension and diabetes is frequent.⁴ Importantly, hypertension in patients with diabetes causes a significant increase in the risk of vascular complications and both conditions predispose to chronic kidney disease.^{5,6} To prevent associated microvascular and macrovascular morbidity and mortality both hypertension and diabetes should be diagnosed early and treated aggressively.⁷⁻¹⁰ Polytherapy requires for proper management of these cases but this increases the risk of drug-drug interactions. Pharmacodynamic interactions affect either the pharmacologic efficacy or the magnitude of side effects of a drug without affecting its plasma levels.¹¹

Sulfonylurea drugs like glibenclamide used for the treatment of Type 2 DM acts by closing ATP sensitive K^+ channels and thus result in increased Ca^{2+} influx and therefore increase insulin release in pancreatic the β - cell, hence decreasing blood glucose level.¹²

Amlodipine is a long acting 1, 4 dihydropyridine derivative Calcium channel antagonist, which is frequently used for the management of hypertension with or without Type 2 diabetes.¹³ Amlodipine blocks voltage dependent Ca^{2+} channels and inhibits the influx of Ca^{2+} into the cells, not only decreasing the concentration of cytoplasmic Ca^{2+} directly but also decrease the Ca^{2+} release from intracellular stores.¹⁴ An increase in cytosolic calcium is essential for the initiation of insulin secretion by glucose and other nutrients; preventing Ca^{2+} influx by removal of extracellular Ca^{2+} or by pharmacologic blockade of voltage - dependent Ca^{2+} channels may blunt nutrient - induced insulin secretion.

Literature review suggest that concomitant use of calcium channel blockers with Sulfonylurea in patients of Type 2 DM, may reduce their antidiabetic efficacy, while some other studies suggests that Amlodipine therapy has no association with clinically significant changes in routine laboratory parameters like plasma glucose, serum potassium etc.^{15,16}

Hence this study was taken up to investigate the effect of amlodipine on blood glucose level in euglycemic and streptozotocin induced diabetic albino rats and its pharmacodynamic interaction with glibenclamide.

METHODS

The study was carried out in the Department of Pharmacology and Therapeutics, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India. Healthy male Wister rats weighing between 150-250gms were selected. The animals were kept in clean and dry cages with 12:12 hours light-dark cycle at room temperature and humidity. They were allowed to acclimatize to the available housing condition for 2 weeks before the initiation of experiment and were fed with standard laboratory diets and water ad libitum.

Drugs used

- Tab. Amlodipine Besylate 5mg (Amlopress), Cipla Limited, Kumrek, India.
- Tab. Glibenclamide 5mg (Daonil) Sanofi India Limited, Ankleshwar.
- Nicotinamide 100gm powder, Animed, Kolkata
- Streptozotocin 250mg powder, HIMEDIA, Mumbai

Induction of diabetes

Diabetes was induced by single intraperitoneal injection nicotinamide (120/kg) followed by injection of freshly prepared streptozotocin (60mg/kg) after 15 minutes.

Nicotinamide prevents complete destruction of pancreatic beta cells by streptozotocin and thereby produces condition similar to Type 2 DM.¹⁷

Following 72 hrs of induction, diabetes was confirmed by measuring the fasting blood glucose (FBG) level. The rats having FBG levels between 250 - 300mg/dl were selected for this study.

Group allocation and drug treatment

Study rats were divided into six groups with 6 animals in each. Dose calculation was done by multiplying the standard human dose with the conversion factor for rat (0.018) to get the desired dose for 200gm. rat.^{18,19} Doses for each rat were then individualized as per their body weight. Drugs were administered orally with help of a gavage tube once daily for 28 days between 08:00-9:30 a.m.

Table 1: Grouping and dosages.

Groups	Drug used	Rat doses
Non diabetic (Control)	1% gum acacia	10ml/kg
Diabetic (Control)	1% gum acacia	10ml/kg
Non diabetic (Amlodipine)	Amlodipine	5mg/kg
Diabetic (Glibenclamide)	Glibenclamide	5mg/kg
Diabetic (Amlodipine)	Amlodipine	5mg/kg
Diabetic (Amlodipine+ Glibenclamide)	Amlodipine+ Glibenclamide	5+5mg/kg

For estimation of FBG rats were kept overnight fasting with free access to water and measured before the induction of diabetes as well as on day 0, 7th, 14th, 21st, and 28th of the study. Blood samples were collected from the tail vein of rats and FBG was measured with the help of Glucometer (Gluco-check), Care bio medical, Mumbai.

Statistical analysis

All the data were expressed as mean \pm SD and were analyzed by using one way ANOVA followed by Post hoc analysis using Tukey's HSD (honestly significant difference) test. P value <0.05 was considered statistically significant.

RESULTS

Table 2 shows there are no significant difference in the FBG level between Group 1 and 3 throughout the study period.

Table 3 shows significant increase in FBG level in Group 5 in comparison to Group 2 on 21st and 28th day of study p value (<0.05).

Table 2: Comparing Mean FBG between Group 1 (Non-Diabetic control) and Group 3 (Non-Diabetic with Amlodipine).

Fasting blood glucose	Group 1	Group 3	Mean difference	P value
Day 0	79.17± 4.79	78.17± 6.08	1.00000	1.000
Day 7	85.33± 3.39	87.17± 5.60	1.83333	0.993
Day 14	84.17± 2.32	88.83± 5.49	4.66667	0.694
Day 21	83.50± 2.59	87.67± 3.14	4.16667	0.680
Day 28	81.17± 3.54	86.50± 2.43	5.33333	0.243

Table 3: Comparing Mean FBG between Group 2 (Diabetic control) and Group 5 (Diabetic with Amlodipine).

Fasting blood glucose	Group 2	Group 5	Mean difference	P value
Day 0	258.33± 7.45	262.17± 6.18	3.83333	0.914
Day 7	268.67± 9.54	271.50± 5.17	2.83333	0.954
Day 14	271.33± 8.09	276.67± 4.76	5.33333	0.565
Day 21	274.17± 9.04	283.50± 3.27	9.33333	0.027
Day 28	278.67± 7.15	290.83± 4.67	12.16667	0.000

Table 4: Comparing Mean FBG between Group 2 (Diabetic control) and Group 4 (Diabetic with Glibenclamide).

Fasting blood glucose	Group 2	Group 4	Mean difference	P value
Day 0	258.33± 7.45	260.33± 8.33	002.00000	0.995
Day 7	268.67 ± 9.54	93.83± 3.66	174.83333	0.000
Day 14	271.33± 8.09	90.33± 5.16	181.00000	0.000
Day 21	274.17± 9.04	80.17± 1.47	194.00000	0.000
Day 28	278.67± 7.15	79.33± 2.34	199.33333	0.000

Comparison of Group 4 rats which were treated with glibenclamide with Group 2 shows consistency in reducing FBS level throughout the study period as depicted in Table 4. In comparison to Group 4 rats,

Group 6 has showed significant increase in FBG level on day 21st and 28th as depicted in Table 5.

Table 5: Comparing Mean FBG between Group 4 (Diabetic with Glibenclamide) and Group 6 (Diabetic with Amlodipine + Glibenclamide).

Fasting blood glucose	Group 4	Group 6	Mean difference	P Value
Day 0	260.33± 8.33	265.67± 6.41	5.33333	0.731
Day 7	93.83± 3.66	97.50± 4.76	3.66667	0.874
Day 14	90.33± 5.16	99.83± 5.88	9.50000	0.060
Day 21	80.17± 1.47	108.17± 5.64	28.00000	0.000
Day 28	79.33± 2.34	120.67± 1.97	41.00000	0.000

DISCUSSION

Due to common existence of diabetes and hypertension, oral hypoglycemic agents are often used along with antihypertensive drugs. Amlodipine is one of the frequently used drug for the management of hypertension. Among the sulfonylureas glibenclamide showed greater increase in insulin sensitivity than other drugs of the same class.²⁰ About interaction between Glibenclamide and Amlodipine, opinions among different literatures are varied.

Similar to the study reported by B. Kishore Kumar Reddy et al. and Wofford MR, King DS et al. current study also showed no increase in FBG level due to Amlodipine in non-diabetic rats.^{16,20} however it contradicts with the findings of Naidu et al.²¹

Normal level of FBG was noted in Glibenclamide treated on 7th day and the result was persistent throughout the study period. However, amlodipine and glibenclamide combination shows initial lowering of FBG level but raised values were observed on 21st and 28th day. This indicates probable pharmacodynamic interaction between amlodipine and glibenclamide in combination group.

Current study shows concomitant use of amlodipine with glibenclamide in the diabetic rats has blunting effect on the blood glucose lowering efficacy of glibenclamide, which was similar to the study reported by Prajapat R et al, Reddy KJ et al.^{22,23}

Blunting of hypoglycemic effect of glibenclamide due to amlodipine may have following probable mechanism - Glibenclamide act by inhibiting ATP sensitive K⁺ channels in the beta cells of pancreas. Inhibition of these K⁺ channels induce depolarization of β cell membrane - which opens voltage sensitive Ca²⁺ channels leading to

influx of calcium ions which stimulates the release of insulin.¹² Amlodipine by blocking the Ca²⁺ channels in pancreatic beta cells may interfere with the insulin release which may cause hyperglycemia.²⁴ In normal rats blockage of Ca²⁺ channel by using amlodipine may not produce hyperglycemia, decrease insulin secretion as the pancreas has healthy β cell. It requires only 30% functioning β - cell to maintain blood sugar level within normal limits. In our experimental model Type 2 diabetes is produced by using nicotinamide + streptozotocin injection which causes only partial destruction of pancreatic β - cell pool. Diabetic rats treated with only amlodipine did not show worsening of hyperglycemia in the initial 2 weeks, may be due to the presence of residual healthy pancreatic β - cells. But as the time progresses worsening of hyperglycemia that was observed could have been contributed by development of insulin resistance or down regulation of insulin receptors.

CONCLUSION

From the results of the study we can conclude that although amlodipine has no significant effect on the fasting blood glucose level in normal rats but it can cause significant worsening of hyperglycemia in diabetic rats. The co-administration of amlodipine with glibenclamide to the diabetic rats has blunting effect on the blood glucose lowering efficacy of glibenclamide. Current study warrants further work to find out the molecular level of interaction between these two drugs and the effect in actual clinical setting need to be explored.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee of Rajendra Institute of Medical Sciences, (RIMS), Ranchi

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